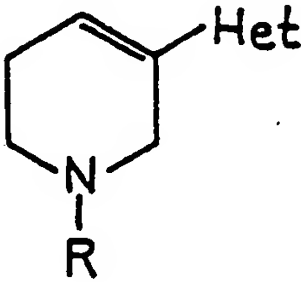




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<p>(21) International Application Number: PCT/US91/08484</p> <p>(22) International Filing Date: 13 November 1991 (13.11.91)</p> <p>(30) Priority data: 620,841 29 November 1990 (29.11.90) US</p> <p>(71) Applicant: ALLERGAN, INC. [US/US]; 2525 Dupont Drive, Post Office Box 19534, Irvine, CA 92713-9534 (US).</p> <p>(72) Inventors: WOLDE MUSSIE, Elizabeth ; 469 Fair Drive, #201, Costa Mesa, CA 92626 (US). CHEN, June ; 28771 Via Los Arboles, San Juan Capistrano, CA 92675 (US). RUIZ, Guadalupe ; 1068 Gentle Drive, Corona, CA 91720 (US).</p>	<p>(74) Agents: BARAN, Robert, J. et al.; Allergan, Inc., 2525 Dupont Drive, Post Office Box 19534, Irvine, CA 92713-9534 (US).</p> <p>(81) Designated States: AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CI (OAPI patent), CM (OAPI patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL (European patent), NO, PL, RO, SD, SE (European patent), SN (OAPI patent), SU⁺, TD (OAPI patent), TG (OAPI patent).</p> <p>Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>	
<p>(54) Title: USE OF PYRIDINE DERIVATIVES IN THE TREATMENT OF OCULAR HYPERTENSION</p> <div style="text-align: center;">  <p>(I)</p> </div> <p>(57) Abstract</p> <p>The present invention relates to a method of treating ocular hypertension by applying to the eye an amount sufficient to treat ocular hypertension of a compound of formula (I), wherein R is hydrogen or an acyclic hydrocarbon group of 1 to about 6 carbon atoms, and Het is a five membered heterocyclic ring containing two or three hetero-atoms selected from N, O and S, unsubstituted or substituted with an acyclic hydrocarbon group of up to about 10 carbon atoms, an alicyclic hydrocarbon group of about 3 to about 6 carbon atoms, or with an -OR₁ group in which R₁ is an acyclic hydrocarbon group of 1 to about 6 carbon atoms, or a pharmaceutically acceptable acid addition salt thereof.</p>		

+ DESIGNATIONS OF "SU"

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USE OF PYRIDINE DERIVATIVES IN THE TREATMENT OF OCULAR HYPERTENSION

Field of the Invention

The present invention relates to the use of certain
5 pyridine derivatives in the treatment of ocular
hypertension.

Background of the Invention

Ocular hypotensive agents are useful in the treatment of
10 a number of various ocular hypertensive conditions, such as
post-surgical and post-laser trabeculectomy ocular
hypertensive episodes, glaucoma, and as presurgical
adjuncts.

Glaucoma is a disease of the eye characterized by
15 increased intraocular pressure. On the basis of its
etiology, glaucoma has been classified as primary or
secondary. For example, primary glaucoma in adults may be
either open-angle or acute or chronic angle-closure.
Secondary glaucoma results from pre-existing ocular diseases
20 such as uveitis, intraocular tumor or an enlarged cataract.

Primary open angle glaucoma is a disease of the optic
nerve which is associated with an increase in intraocular
pressure (IOP). This increase is due to increased
resistance to aqueous humor outflow at the trabecular
25 meshwork [Johnson, D.H. and Brubaker, R.F.: Glaucoma: An
overview. Mayo Clin. Proc., G1:59-67 (1986)].

In chronic open-angle glaucoma, the anterior chamber and
its anatomic structures appear normal, but drainage of the
aqueous humor is impeded. In acute or chronic angle-closure
30 glaucoma, the anterior chamber is shallow, the filtration
angle is narrowed, and the iris may obstruct the trabecular
meshwork at the entrance of the canal of Schlemm. Dilation
of the pupil may push the root of the iris forward against
the angle, and may produce pupillary block and thus
35 precipitate an acute attack. Eyes with narrow anterior
chamber angles are predisposed to acute angle-closure
glaucoma attacks of various degrees of severity.

Secondary glaucoma is caused by any interference with
the flow of aqueous humor from the posterior chamber into
40 the anterior chamber and subsequently, into the canal of

Schlemm. Inflammatory disease of the anterior segment may prevent aqueous escape by causing complete posterior synechia in iris bombe, and may plug the drainage channel with exudates. Other common causes are intraocular tumors, enlarged cataracts, central retinal vein occlusion, trauma to the eye, operative procedures and intraocular hemorrhage.

Considering all types together, glaucoma occurs in about 2% of all persons over the age of 40 and may be asymptotic for years before progressing to rapid loss of vision.

One drug that has been used for the treatment of glaucoma for over a hundred years is pilocarpine [(3S,4R)-3-ethyl-4-((1-methyl-1H-imidazol-5-yl)methyl)-3,4-dihydro-2(3H)-furanone]. Pilocarpine is a muscarinic agonist that reduces resistance to aqueous humor outflow by causing contraction of the ciliary muscle, thus pulling the trabecular meshwork apart. [See, e.g. Barany, E.H., Invest. Ophthalmol. 1, 712-727 (1962); and Kaufman, P.L. and Barany, E.H., Invest. Ophthalmol. 15, 793-807 (1976).] Although pilocarpine is a safe and effective drug, it has undesired side effects such as miosis (reduction of pupil size) due to contraction of the iris sphincter muscle. It also has short duration of action, and therefore, has to be administered four-times a day which creates a problem with patient compliance. These undesired properties have limited the wide application of pilocarpine and other muscarinics, and in the past decade other compounds with different mechanism of action such as inhibition of aqueous humor formation, have been the drugs of choice.

A well known compound acting via reduction of the production of the aqueous humor is epinephrine (also called adrenaline), by its most commonly used chemical name 4-[1-hydroxy-2-(methylamino)ethyl]-1,2-benzenediol. Its 1-form has adrenergic properties, and together with its dipivalate ester derivative, propine, is a clinically useful anti-glaucoma agent. However, these compounds also cause mydriasis, an excessive dilation of the pupil of the patient's eye upon administration.

Certain eicosanoids and their derivatives have been reported to possess ocular hypotensive activity, and have been recommended for use in glaucoma management.

Eicosanoids and derivatives include numerous biologically important compounds such as prostaglandins and their derivatives.

5 Certain compounds, structurally similar to the muscarinic agonist arecoline [1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxylic acid methyl ester] were disclosed in the European Patent Applications Publication Nos. 259,621; 296,721 and 316,718 as suitable for the treatment of cognitive disorders such as Alzheimer's disease.

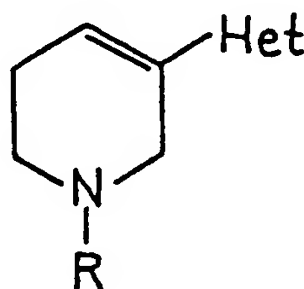
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Summary of the Invention

In search of muscarinic agonists, we have identified a group of compounds which effectively reduce ocular pressure but are substantially devoid of the undesired side effects of pilocarpine and its analogs. These compounds are encompassed by the formula (I)

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where R is hydrogen or an acyclic hydrocarbon group of about 1 to 6 carbon atoms, and Het is a five membered heterocyclic ring containing two or three hetero-atoms selected from N, O and S, unsubstituted or substituted with an acyclic hydrocarbon group of up to about 10 carbon atoms, or an alicyclic hydrocarbon group of about 3 to 6 carbon atoms, or with an -OR₁ group in which R₁ is an acyclic hydrocarbon group of 1 to about 6 carbon atoms. These and structurally related compounds were disclosed in EP 296,721 (US 4,866,077), EP 316,718 and EP 259,621 as structural analogs of arecoline, a cholinergic agonist. Since the disclosed compounds were described as having potent acetylcholine activity indicating utility in the treatment of diseases caused by reduced function of acetylcholine in the brain, in particular, Alzheimer's disease, their ocular hypotensive activity is highly unexpected.

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In one aspect, the present invention relates to a method of treating ocular hypertension which comprises applying to

the eye an amount sufficient to treat ocular hypertension of a compound of the above formula (I), wherein the substituents are as hereinabove defined, or a pharmaceutically acceptable acid addition salt thereof.

5 In another aspect, the invention relates to an ophthalmic solution for the treatment of ocular hypertension, comprising an amount sufficient to treat ocular hypertension of a compound of formula (I) as hereinabove defined, or a pharmaceutically acceptable acid addition salt thereof, in admixture with a non-toxic, 10 ophthalmically acceptable liquid vehicle, packaged in a container suitable for metered application.

In a further aspect, the invention relates to a pharmaceutical product, comprising

15 a container adapted to dispense its contents in metered form; and

an ophthalmic solution therein, as hereinabove defined.

Brief Description of the Figures

20 Figure 1 illustrates the effect of pilocarpine on intraocular pressure (IOP) in rabbits. Pilocarpine (10 μ g/20 μ l) (\square) or saline (o) was injected intracamerally and IOP was measured at indicated times.

Figure 2 shows the effect of pyridine-1,2,3,6-tetrahydro-5-(5-isoxazolyl)-1-methyl-ethanedioate (test 25 compound) on IOP in rabbits. The test compound (10 μ g/20 μ l) (\bullet) or saline (o) was injected intracamerally and IOP was measured at indicated times.

Figure 3 shows the effect of pilocarpine on IOP in owl 30 monkeys. Pilocarpine (\square) (200 μ g/10 μ l) was applied topically. Control animals received saline (o).

Figure 4 illustrates the effect of the test compound on IOP in owl monkeys. The test compound (200 μ g/10 μ l) (\bullet) was applied topically. Control animals received saline (o).

35 Figure 5 shows the effect of atropine on the test compound-induced decrease in IOP in rabbits. Atropine (5 μ g/20 μ l) was injected with the test compound (10 μ g/20 μ l) (\square) and IOP was measured. Values are compared with the test compound alone (\bullet) or saline (o).

Figure 6 shows the effect of atropine on the test compound-induced reduction in IOP in owl monkeys. Atropine was applied topically ($5 \mu\text{g}/10 \mu\text{l}$) (\square). Values are compared with the test compound alone ($200 \mu\text{g}/10 \mu\text{l}$) (\cdot) or saline (o).

Figure 7 illustrates the decrease in pupil size induced by intracamerally applied pilocarpine ($10 \mu\text{g}/20 \mu\text{l}$) (\square). Control animals received saline (o).

Figure 8 shows the decrease in pupil size in rabbits induced by intracamerally applied test compound ($10 \mu\text{g}/20 \mu\text{l}$) (\cdot). Control animals received saline (o).

Figure 9 shows the effect of pilocarpine on pupil size in owl monkeys. Pilocarpine ($200 \mu\text{g}/\mu\text{l}$) (\square) was applied topically. Control animals received saline (o).

Figure 10 illustrates the effect of the test compound on pupil size in owl monkeys. The test compound ($200 \mu\text{g}/10 \mu\text{l}$) (\cdot) was applied topically. Control animals received saline (o).

Figure 11 shows the effect of atropine on the test compound-induced miosis in rabbits. Pupil diameter was measured at indicated times following intracameral injection of $10 \mu\text{g}/20 \mu\text{l}$ test compound (\square) or test compound and $5 \mu\text{g}$ atropine (\cdot). Saline (o) was injected into control animals.

Figure 12 shows the effect of atropine on test compound-induced miosis in owl monkeys. Atropine (\square) ($5 \mu\text{g}/10 \mu\text{l}$) was applied topically. Values are compared to the test compound alone (\cdot) or saline (o).

Figure 13 shows the effect of the test compound on contraction of cat ciliary (\square) or iris sphincter (\cdot) smooth muscle.

Figure 14 illustrates the effect of atropine (open symbols) on test compound-induced contraction of cat ciliary or iris sphincter smooth muscle. Tissues were incubated with 10nM atropine as described in the Examples. Test compound-induced contraction is shown by closed symbols.

Detailed Description of the Invention

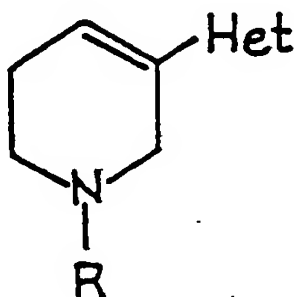
The present invention is based on the unexpected finding that certain analogs of the muscarinic agonist arecoline

cause a reduction in intraocular pressure with minimal effect on pupil size, and have a long duration of action.

The compounds used in accordance with the present invention have the following structure

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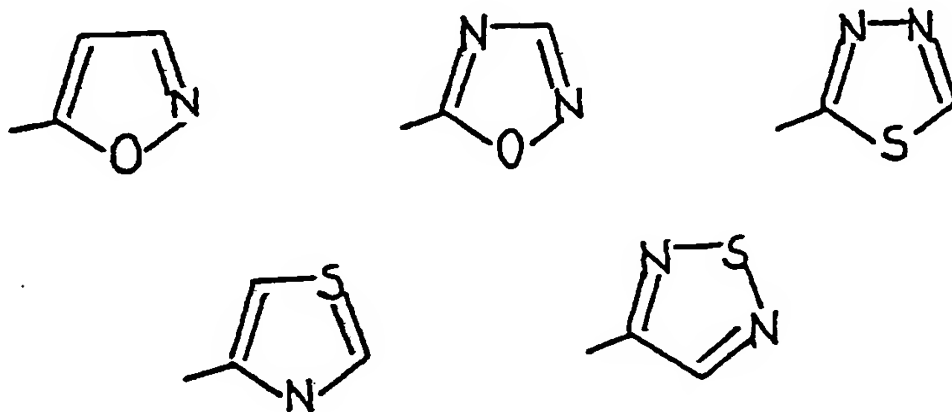
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wherein R is hydrogen or an acyclic hydrocarbon group of 1 to about 6 carbon atoms, and Het is a five membered heterocyclic ring containing two or three hetero-atoms selected from N, O and S, unsubstituted or substituted with an acyclic hydrocarbon group of up to about 10 carbon atoms, or an alicyclic hydrocarbon group of about 3 to 6 carbon atoms, or with an -OR₁ group in which R₁ is an acyclic hydrocarbon group of 1 to about 6 carbon atoms.

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Het as a five membered heterocyclic group may include one or two double bonds, and preferably is selected from

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In the above substituent definitions, the term "acyclic hydrocarbon group" can be saturated or unsaturated, and is used to refer to a straight or branched chained saturated or unsaturated hydrocarbon groups of appropriate lengths, including straight and branched chained alkyl, alkenyl and

alkinyl groups. Within this definition, preferred are the alkyl groups, preferably having 1 to about 6 carbon atoms, such as methyl, ethyl, n- and i-propyl, n- and t-butyl, n- and i-pentyl, n- and i-hexyl group, etc. R preferably is an alkyl group having 1 to 4 carbon atoms, more preferably 1 or 2 carbon atoms. In the most preferred compounds of formula (I) R is a methyl group. In the definition of R₁, the preferred acyclic hydrocarbon groups are those having up to 4 carbon atoms.

10 The term "alicyclic hydrocarbon group" is used to refer to a saturated or unsaturated cyclic hydrocarbon group having from 3 to about 6 carbon atoms, and includes cyclopropyl, cyclopentyl, cyclopentenyl, cyclohexyl and cyclohexenyl groups, etc.

15 In a particularly preferred group of the compounds according to the present invention the Het heterocyclic ring is an isoxazole, thiazole, 1,2,4-oxadiazole, 1,3,4-thiadiazole or 1,2,5-thiadiazole ring.

Particularly preferred are the following compounds:

20 pyridine-1,2,3,6-tetrahydro-5-(5-isoxazolyl)-1-methyl-ethanedioate;

pyridine-1,2,3,6-tetrahydro-5-[3-(n-butyl)-1,2,4-oxadiazol-5-yl]-1-methyl-ethanedioate;

25 pyridine-1,2,3,6-tetrahydro-5-[3-(n-heptyl)-1,2,4-oxadiazol-5-yl]-1-methyl-ethanedioate;

pyridine-1,2,3,6-tetrahydro-5-(3-propyl-1,2,4-oxadiazol-5-yl)-ethanedioate;

pyridine-1,2,3,6-tetrahydro-5-(2-methoxy-1,3,4-thiadiazol-5-yl)-1-methyl-ethanedioate;

30 pyridine-1,2,3,6-tetrahydro-5-(2-methyl-4-thiazolyl)-1-methyl-ethanedioate;

pyridine-1,2,3,6-tetrahydro-5-(3-methoxy-1,2,5-thiadiazol-4-yl)-1-methyl-ethanedioate;

35 and the pharmaceutically acceptable acid addition salts of these compounds.

The pharmaceutically acceptable acid addition salts of the compounds of formula (I) contain an anion which is not toxic in usual therapeutic doses. Preferred acid addition salts include hydrochlorides, hydrobromides, sulphates, acetates, phosphates, nitrates, methanesulphonates,

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ethanesulphonates, lactates, citrates, tartarates, etc. Further acids suitable for forming pharmaceutically acceptable acid addition salts are, for example, fumaric, benzoic, succinic, palmitic, benzenesulphonic, etc. acids.

5 The compounds of the formula (I) are either specifically disclosed in one or more of the above-mentioned European Patent Applications, or can be prepared from known compounds by methods known in the art of organic chemistry.

10 The disclosed compounds exhibit valuable pharmaceutical properties. More particularly, these compounds are potent anti-glaucoma agents that are essentially devoid of the undesirable side-effects of the known muscarinic agonist anti-glaucoma agents currently used in clinical practice, such as pilocarpine. Moreover, these compounds have a
15 significantly longer duration of action.

Pharmaceutical compositions may be prepared by combining a therapeutically efficient amount of at least one compound according to the present invention, or a pharmaceutically acceptable acid addition salt thereof, as an active
20 ingredient, with conventional pharmaceutical excipients. Preferably, at least one of the active ingredients is a compound of formula (I), wherein the substituents are as hereinabove defined. The therapeutically efficient amount typically is between about 0.1 and about 5 % (w/v) in liquid
25 formulations.

For ophthalmic application, preferably solutions are prepared using a physiological saline solution as a major vehicle. The pH of such ophthalmic solutions should preferably be maintained between 6.5 and 7.2 with an
30 appropriate buffer system. The formulations may also contain conventional, pharmaceutically acceptable preservatives and stabilizers.

Preferred preservatives that may be used in the pharmaceutical compositions of the present invention
35 include, but are not limited to, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate and phenylmercuric nitrate. Likewise, various preferred vehicles may be used in the ophthalmic preparations of the present invention. These vehicles include, but are not
40 limited to, polyvinyl alcohol, povidone, hydroxypropyl

methvl cellulose, poloxamers, carboxymethyl cellulose, hydroxyethyl cellulose and purified water.

Tonicity adjustors may be added as needed or convenient. They include, but are not limited to, salts, particularly sodium chloride, potassium chloride, mannitol and glycerin, or any other suitable ophthalmically acceptable tonicity adjustor.

Various buffers and means for adjusting pH may be used so long as the resulting preparation is ophthalmically acceptable. Accordingly, buffers include acetate buffers, citrate buffers, phosphate buffers and borate buffers. Acids or bases may be used to adjust the pH of these formulations as needed.

In a similar vein, an ophthalmically acceptable antioxidant for use in the present invention includes, but is not limited to, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene.

Other excipient components which may be included in the ophthalmic preparations are chelating agents. The preferred chelating agent is edetate disodium, although other chelating agents may also be used in place or in conjunction with it.

The ingredients are usually used in the following amounts:

<u>Ingredient</u>	<u>Amount (% w/v)</u>
active ingredient	about 0.1-5
preservative	0-0.10
vehicle	0-40
tonicity adjustor	0-10
buffer	0.01-10
pH adjustor	q.s. pH 4.5-7.5
antioxidant	as needed
purified water	as needed to make 100%

The pharmacological activity of the compounds according to the present invention is further illustrated by the following non-limiting Examples.

Example 1

In vivo study of intraocular pressure reducing activity.

Methods

Pyridine-1,2,3,6-tetrahydro-5-(5-isoxazolyl)-1-methyl-ethanedioate ("test compound") or pilocarpine were applied either topically into the eyes of owl monkeys (200 μ g in 10 μ l) or intracamerally into the eye of rabbits (10 μ g in 20 μ l). Control animals were treated with a saline vehicle.

Pupil diameter and IOP were measured for 0-6 hours. IOP was measured using pneumatometer (Digilab/ Biorad) and pupil diameter was measured using Optistick ruler (Allergan). Animals used in these studies had normal intraocular pressure.

Muscarinic activity was determined by treatment with atropine (muscarinic antagonist). In rabbits 5 μ g atropine was coinjected with the test compounds and in owl monkey 5 μ g atropine (10 μ l) was applied 30 minutes before treatment with the test compound.

Intraocular pressure (mmHg) or pupil diameter (mm) were expressed as changes from time 0.

Results and Discussion

Intraocular pressure. Muscarinic agonists such as pilocarpine cause little or no decrease in IOP in normotensive rabbits or humans [Miichi, H. and Nagataki, S., J. ophthalmol. 26, 4250436 (1982); Naveh-Floman et al., Ophthalmic Res. 18, 34-37 (1986)]. The owl monkey is one of the few species that responds to pilocarpine [Lamble, et al., Invest. Ophthalmol. 15, 848-851 (1976)].

The results presented in Figure 1 confirm this observation on pilocarpine's effect in rabbits. Pilocarpine (10 μ g/20 μ l) injected intracamerally showed no hypotensive effect. In fact, as shown in Figure 1, there was a slight hypertensive action.

This study also shows that the test compound differs from pilocarpine with respect to its effect on IOP in rabbits. When applied intracamerally, the test compound caused decrease in IOP of 6 mmHg 5 hours after administration (Figure 2). This activity appeared to last longer than 6 hours which was the end of the experimental period. This showed that not only did the test compound cause a decrease in IOP, but also had a longer duration of action.

As mentioned above, the owl monkey is one of the few normotensive animals that respond to pilocarpine. As expected, pilocarpine caused a decrease in IOP of about 3 mmHg at 3 hours and the effect decreased thereafter (Figure 3).

Application of the test compound into the eyes of owl monkeys also caused reduction in IOP with maximum decrease of 4 mmHg after 5 and 6 hours (Figure 4). Here again, as in the case of rabbits, the effect lasted longer than the experimental period of 6 hours, indicating that the test compound has a longer duration than that of pilocarpine in the owl monkey.

An observation of such an effect for a muscarinic agonist is the first of its kind, especially in normotensive rabbits. Thus, the next question was whether the test compound caused reduction in IOP by activating muscarinic receptors. This was investigated by pretreating the animals with atropine, a muscarinic antagonist. In rabbits, coinjection of atropine with the test compound only caused partial inhibition of IOP effect (Figure 5). Topical application of a 0.5% atropine solution every 2 hours had no inhibitory effect either (data not shown). Similarly, topical application of 5 µg/10 µl atropine into eyes of owl monkeys had no inhibitory effect on the IOP reducing effect of the test compound (Figure 6). It is important to note that this concentration of atropine is several orders of magnitude higher than what is required to cause complete inhibition of activity on muscarinic receptors. This suggests that the mechanism of action of the test compound for reducing IOP in both owl monkeys and rabbits may not be entirely via muscarinic receptors.

Miosis. Both pilocarpine and the test compound caused miosis in rabbits, but the effect of pilocarpine was greater than that of the test compound (Figures 7 and 8). In both cases, maximum response was obtained at 0.5 hours and it was followed by recovery through the rest of the experimental period.

The miotic effect of pilocarpine was severe in owl monkeys, causing complete closure of the pupil for longer than 24 hours (Figure 9). Miosis caused by the test

compound, on the other hand, was much less with a decrease of 48% at 1 hour which recovered to 15% of 0 time within 3 to 6 hours (Figure 10). The miotic effect of the test compound in both species was completely inhibited by atropine (Figures 11 and 12). This indicates the involvement of muscarinic receptors of the iris sphincter muscle.

Example 2

In vitro studies.

10 Methods

Iris sphincter and ciliary smooth muscles were excised from freshly enucleated cat eyes. The tissues were placed in an organ bath containing Tyrode's solution (composition in mM: NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.05, NaHCO₃ 11.9, NaH₂PO₄ 0.42, D-glucose 5.6) at 37°C, aerated with 95% oxygen and 5% carbon dioxide. One end of the tissue was fastened to a hook in the bath and the other end was attached to a force displacement transducer connected to a polygraph. The tissues were allowed to equilibrate for 60 minutes under a load of 100-200 mg tension. After equilibration, suboptimal concentrations of bethanechol (10-30 µM) were used repeatedly to prime the tissues. Increasing concentrations of the test compound (0.1 - 1000 µM) were then added into the bath to generate cumulative dose response. To test the effect of muscarinic antagonist, the tissues were incubated with 10 nM atropine for 30 minutes before treatment with the test compound.

Agonist activity was expressed as ED₅₀ - a concentration that caused 50% of maximum contraction in a cumulative dose response curve. Results were from three or more experiments expressed as mean ± S.E.

Results and Discussion

This study was done to quantitatively evaluate the activity of pyridine-1,2,3,6-tetrahydro-5-(5-isoxazolyl)-1-methyl-ethanedioate ("test compound") on the ciliary and iris sphincter smooth muscles in order to understand the mechanism of action of the compound. Previous studies have shown that muscarinic agonists cause a decrease in IOP by contracting the ciliary muscle [Kaufman, P.L. and Barany, E.H., Invest. Ophthalmol. 15, 793-807 (1976)]. Its action on

the iris sphincter muscle causes miosis which is an undesired side effect. In this study, the test compound caused a concentration related contraction of both ciliary and iris sphincter muscles with ED_{50} of 30 and 20 μM , respectively. This compound was less potent than pilocarpine which had ED_{50} of 4 μM in both tissues (Figure 13). Atropine caused competitive inhibition of contraction indicating that this compound activates muscarinic receptors in these tissues (Figures 14 and 15). However, the effect of atropine was 2.5-times greater in inhibiting the response of the iris than that of the ciliary. This appears to agree with the data from the in vivo studies presented in Example 1, whereby miosis was completely and IOP partially affected by atropine.

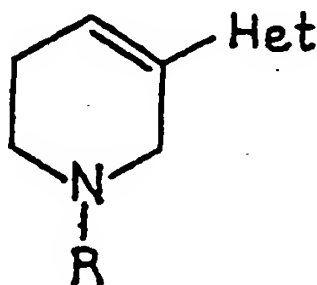
The data presented in Examples 1 and 2 clearly indicate that the test compound caused decrease in IOP in two animal models, i.e. rabbits and owl monkeys. Although such an effect has been previously demonstrated for muscarinic agents in owl monkeys [Lamble, J.W. and Lamble, A.P., Invest. Ophthalmol. 15, 848-851 (1976)], this is the first time that such an observation was made in rabbits. Screening of compounds for use in glaucoma therapy is mostly done using rabbits. Thus the fact that the test compound and its analogs lower IOP in rabbits is an added advantage over pilocarpine. The test compound was also more effective in lowering IOP in owl monkeys although the in vitro studies have shown that it was less potent than pilocarpine. From the in vitro studies it was also clear that these compounds can cause contraction of the ciliary muscle of the cat by an atropine sensitive receptor. However, the lack of complete inhibition of its IOP lowering effect by very high concentrations of atropine suggests that the test compounds may have another mechanism of action of reducing IOP. A non-muscarinic activity of such compounds has not been previously observed.

The ability of the compounds disclosed in the present invention to decrease IOP with long duration and the reduced and short-lived miosis accompanying the IOP reducing activity make these compounds excellent candidates for glaucoma therapy.

The foregoing description details specific formulations and methods that can be employed to practice the present invention. Having detailed specific compositions for the ophthalmic formulations of the present invention and
5 specific instructions for their use in the treatment of ocular hypertension, the art skilled will well enough know how to devise other formulations and how to adapt the treatment (formulations, doses) to a special situation. Thus, however detailed the foregoing may appear in text, it
10 should not be construed as limiting the overall scope hereof; rather, the ambit of the present invention is to be governed only by the lawful construction of the appended claims.

CLAIMS:

1. A method of treating ocular hypertension which comprises applying to the eye an amount sufficient to treat ocular hypertension of a compound of the formula (I)



wherein R is hydrogen or an acyclic hydrocarbon group of 1 to about 6 carbon atoms, and Het is a five membered heterocyclic ring containing two or three hetero-atoms selected from N, O and S, unsubstituted or substituted with an acyclic hydrocarbon group of up to about 10 carbon atoms, an alicyclic hydrocarbon group of about 3 to about 6 carbon atoms, or with an $-OR_1$ group in which R_1 is an acyclic hydrocarbon group of 1 to about 6 carbon atoms, or a pharmaceutically acceptable acid addition salt thereof.

2. The method according to Claim 1 wherein Het is a five membered heterocyclic ring containing oxygen and nitrogen, or sulfur and nitrogen as heteroatom.

3. The method according to Claim 2 wherein Het is selected from the group consisting of isoxazole, thiazole, 1,2,4-oxadiazole, 1,3,4-thiadiazole and 1,2,5-thiadiazole ring.

4. The method according to Claim 3 wherein R is an alkyl group having from 1 to 4 carbon atoms.

5. The method according to Claim 4 wherein R is methyl.

6. The method according to Claim 5 wherein the Het heterocyclic ring is unsubstituted or substituted with an alkyl group having from 1 to about 8 carbon atoms.

7. The method according to Claim 5 wherein said heterocyclic ring is substituted with an alkoxy group having from 1 to about 6 carbon atoms in the alkyl moiety.

8. The method according to Claim 7 wherein said alkoxy group has from 1 to 4 carbon atoms in the alkyl moiety.

9. The method according to Claim 3 wherein R is hydrogen.

10. The method of Claim 9 wherein the Het heterocyclic ring is substituted with an alkyl group having from 1 to about 4 carbon atoms.

11. The method according to Claim 1 wherein said compound of formula (I) is selected from the group consisting of

5 pyridine-1,2,3,6-tetrahydro-5-(5-isoxazolyl)-1-methyl-ethanedioate;

pyridine-1,2,3,6-tetrahydro-5-[3-(n-butyl)-1,2,4-oxadiazol-5-yl]-1-methyl-ethanedioate;

pyridine-1,2,3,6-tetrahydro-5-[3-(n-heptyl)-1,2,4-oxadiazol-5-yl]-1-methyl-ethanedioate;

10 pyridine-1,2,3,6-tetrahydro-5-(3-propyl-1,2,4-oxadiazol-5-yl)-ethanedioate;

pyridine-1,2,3,6-tetrahydro-5-(2-methoxy-1,3,4-thiadiazol-5-yl)-1-methyl-ethanedioate;

15 pyridine-1,2,3,6-tetrahydro-5-(2-methyl-4-thiazolyl)-1-methyl-ethanedioate;

pyridine-1,2,3,6-tetrahydro-5-(3-methoxy-1,2,5-thiadiazol-4-yl)-1-methyl-ethanedioate;

and a pharmaceutically acceptable acid addition salt of any of these compounds.

12. The method according to Claim 11 wherein said compound is pyridine-1,2,3,6-tetrahydro-5-(5-isoxazolyl)-1-methyl-ethanedioate, or a pharmaceutically acceptable acid addition salt thereof.

13. An ophthalmic solution for the treatment of ocular hypertension, comprising an amount sufficient to treat ocular hypertension of a compound of formula (I) as defined in Claim 1, or a pharmaceutically acceptable acid addition salt thereof, in admixture with a non-toxic, ophthalmically acceptable liquid vehicle, packaged in a container suitable for metered application.

14. The ophthalmic solution according to Claim 13 wherein said compound of formula (I) is selected from the group consisting of

5 pyridine-1,2,3,6-tetrahydro-5-(5-isoxazolyl)-1-methyl-ethanedioate;

- pyridine-1,2,3,6-tetrahydro-5-[3-(n-butyl)-1,2,4-oxadiazol-5-yl]-1-methyl-ethanedioate;
pyridine-1,2,3,6-tetrahydro-5-[3-(n-heptyl)-1,2,4-oxadiazol-5-yl]-1-methyl-ethanedioate;
10 pyridine-1,2,3,6-tetrahydro-5-(3-propyl-1,2,4-oxadiazol-5-yl)-ethanedioate;
pyridine-1,2,3,6-tetrahydro-5-(2-methoxy-1,3,4-thiadiazol-5-yl)-1-methyl-ethanedioate;
pyridine-1,2,3,6-tetrahydro-5-(2-methyl-4-thiazolyl)-1-
15 methyl-ethanedioate;
pyridine-1,2,3,6-tetrahydro-5-(3-methoxy-1,2,5-thiadiazol-4-yl)-1-methyl-ethanedioate;
and a pharmaceutically acceptable acid addition salt of any of these compounds.

15. The ophthalmic solution according to Claim 14, wherein said compound is pyridine-1,2,3,6-tetrahydro-5-(5-isoxazolyl)-1-methyl-ethanedioate or a pharmaceutically acceptable acid addition salt thereof.

16. A pharmaceutical product, comprising
a container adapted to dispense its contents in metered form; and

an ophthalmic solution therein, as defined in Claim 13.

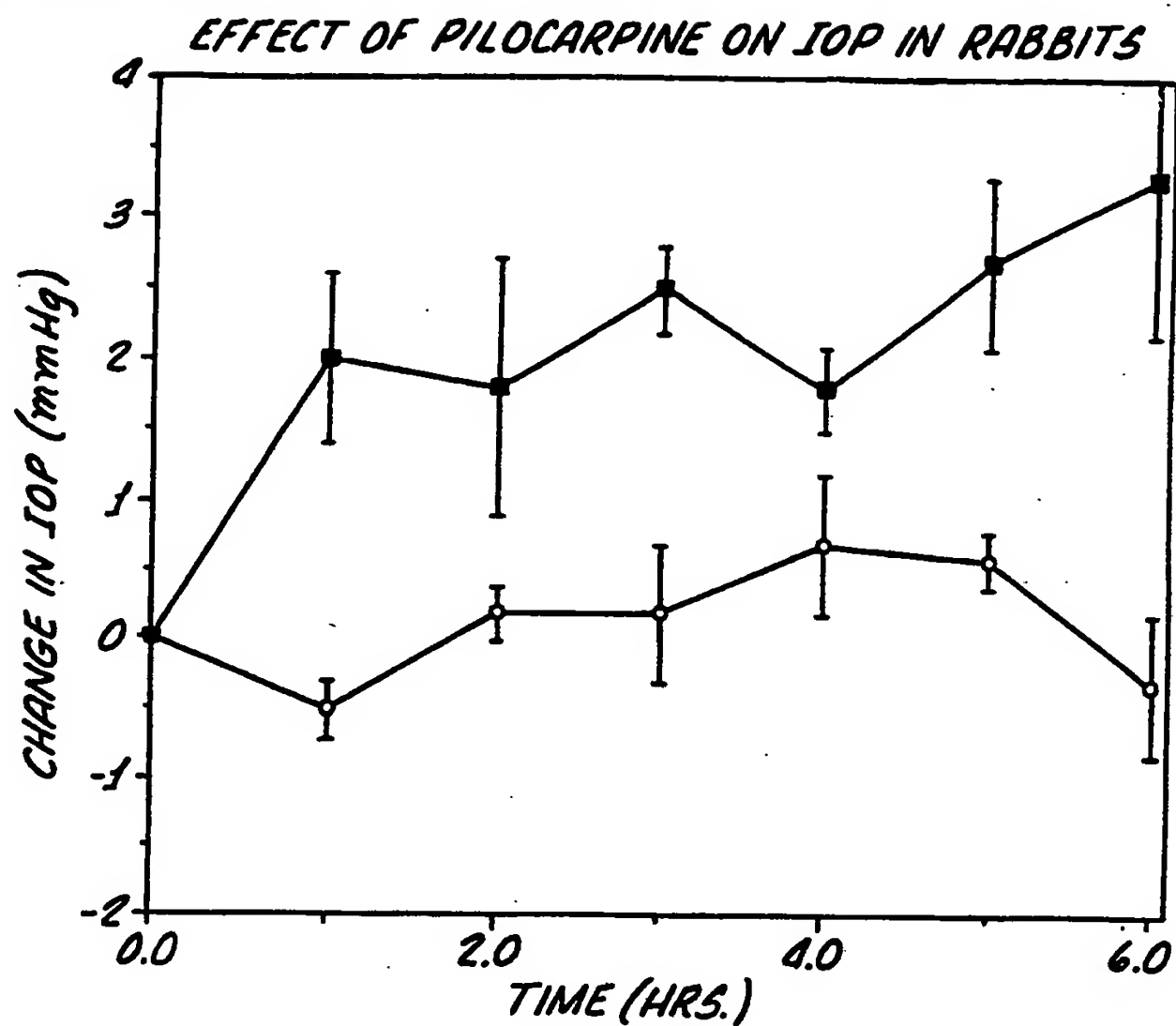
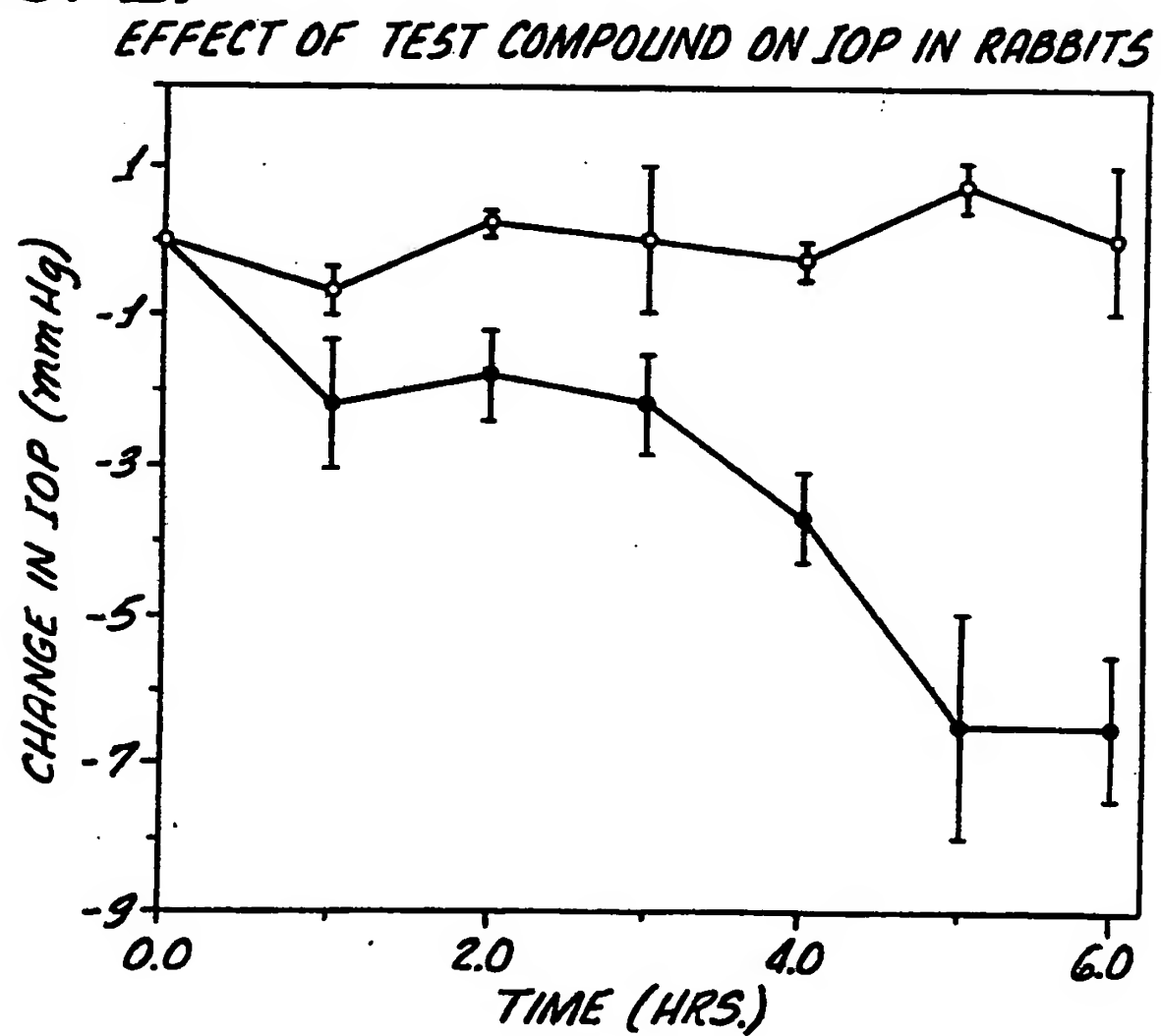
17. The pharmaceutical product according to Claim 16 comprising an ophthalmic solution wherein said compound of formula (I) is selected from the group consisting of
pyridine-1,2,3,6-tetrahydro-5-(5-isoxazolyl)-1-methyl-
5 ethanedioate;
pyridine-1,2,3,6-tetrahydro-5-[3-(n-butyl)-1,2,4-oxadiazol-5-yl]-1-methyl-ethanedioate;
pyridine-1,2,3,6-tetrahydro-5-[3-(n-heptyl)-1,2,4-oxadiazol-5-yl]-1-methyl-ethanedioate;
10 pyridine-1,2,3,6-tetrahydro-5-(3-propyl-1,2,4-oxadiazol-5-yl)-ethanedioate;
pyridine-1,2,3,6-tetrahydro-5-(2-methoxy-1,3,4-thiadiazol-5-yl)-1-methyl-ethanedioate;
pyridine-1,2,3,6-tetrahydro-5-(2-methyl-4-thiazolyl)-1-
15 methyl-ethanedioate;
pyridine-1,2,3,6-tetrahydro-5-(3-methoxy-1,2,5-thiadiazol-4-yl)-1-methyl-ethanedioate;

and a pharmaceutically acceptable acid addition salt of any of these compounds.

18. The pharmaceutical product according to Claim 17 wherein said compound is pyridine-1,2,3,6-tetrahydro-5-(5-isoxazolyl)-1-methyl-ethanedioate or a pharmaceutically acceptable acid addition salt thereof.

FIG. 1.

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*FIG. 2.*

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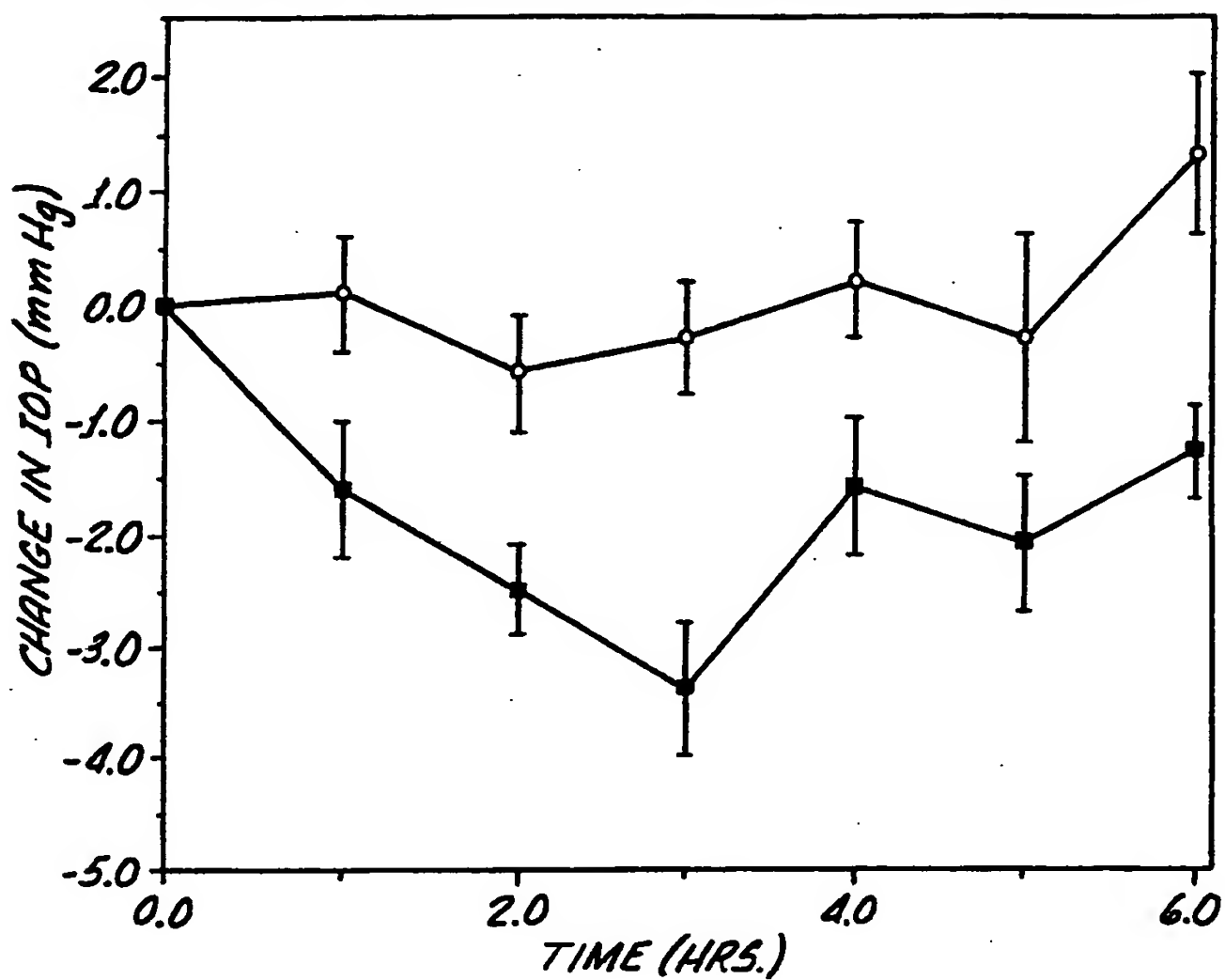
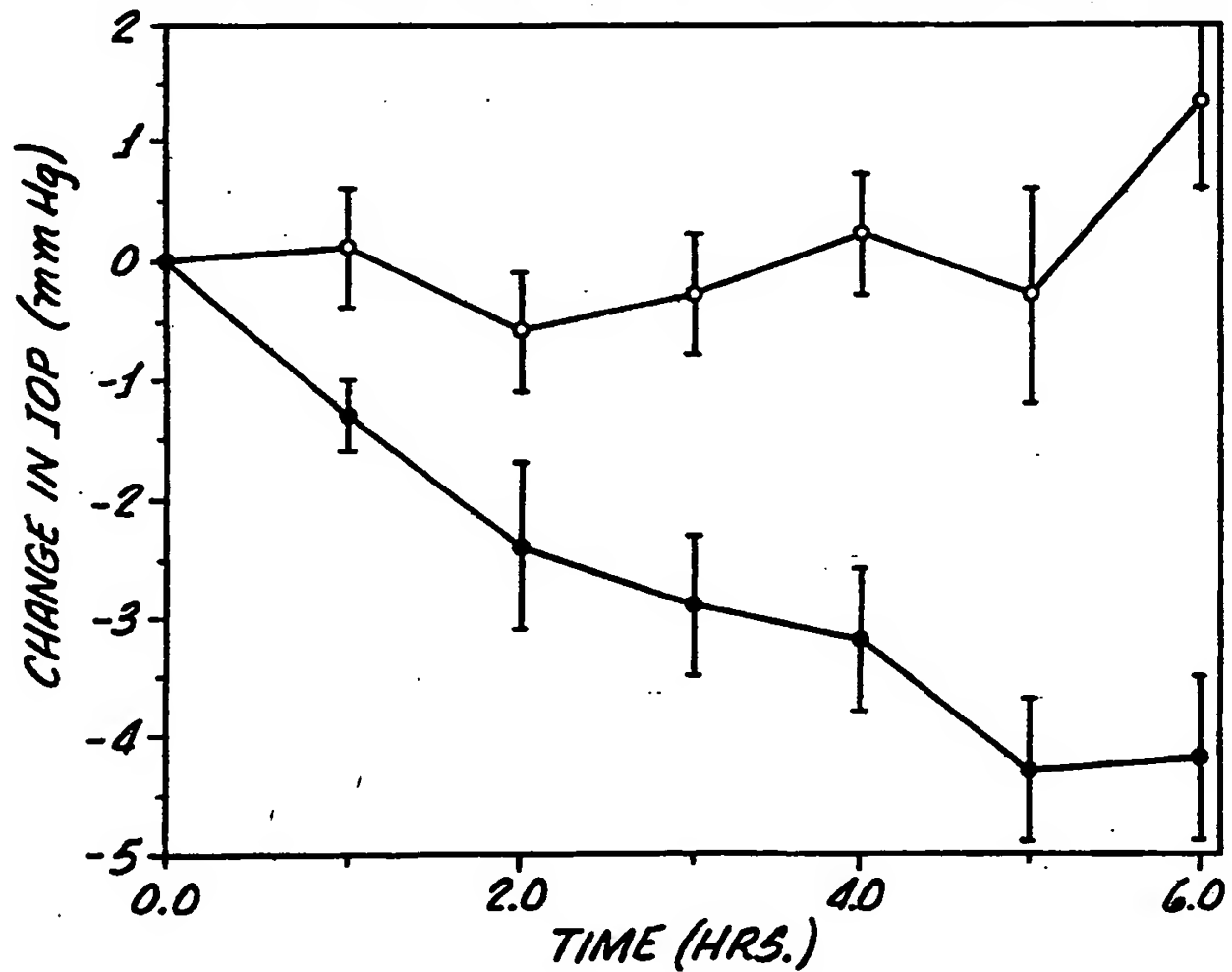
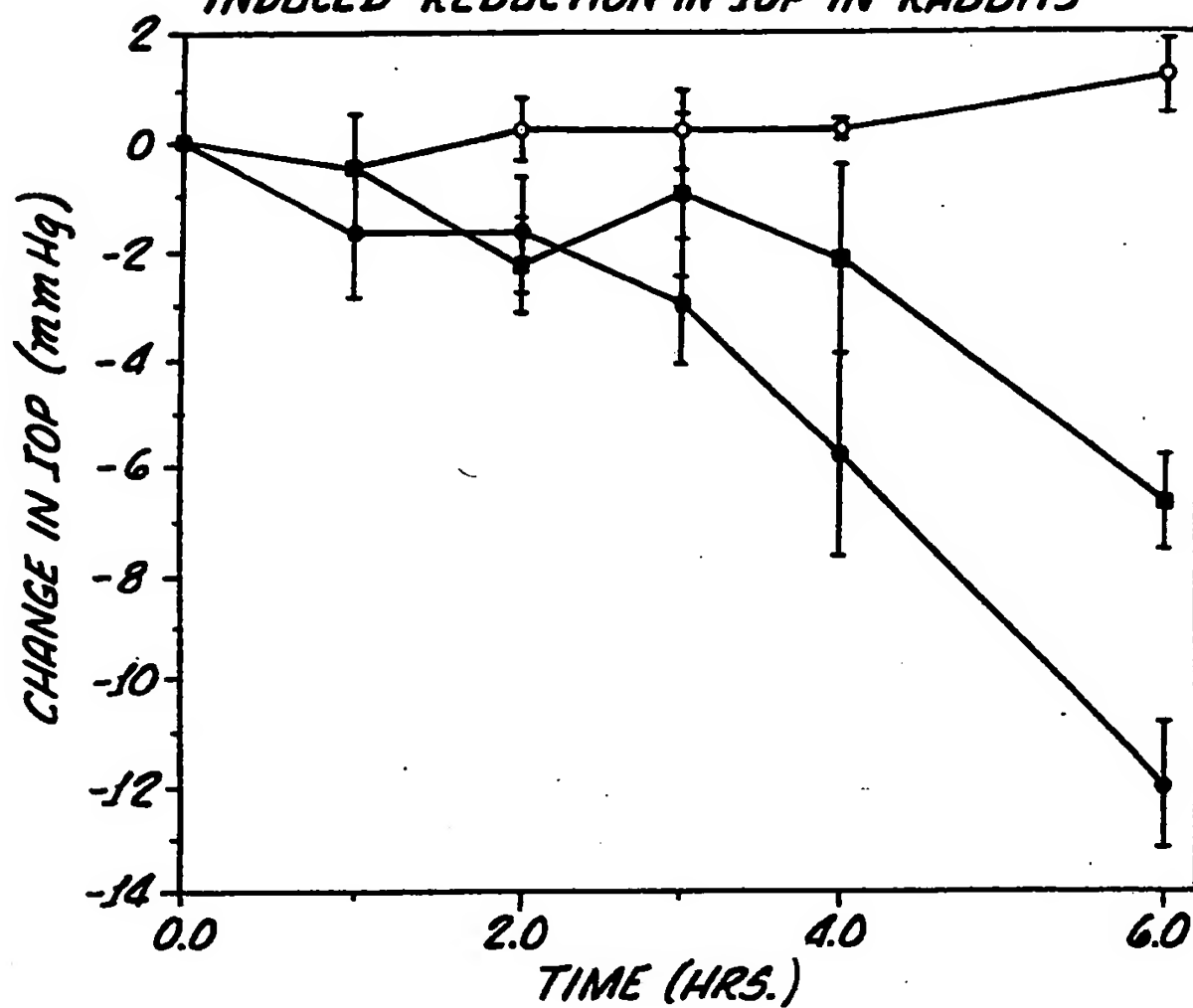
FIG. 3.**EFFECT OF PILOCARPINE ON IOP IN OWL MONKEYS****FIG. 4.****EFFECT OF TEST COMPOUND ON IOP IN OWL MONKEYS**

FIG. 5.

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EFFECT OF ATROPINE ON TEST COMPOUND
INDUCED REDUCTION IN IOP IN RABBITS

FIG. 6.

EFFECT OF ATROPINE ON TEST COMPOUND
INDUCED DECREASE IN IOP IN OWL MONKEYS

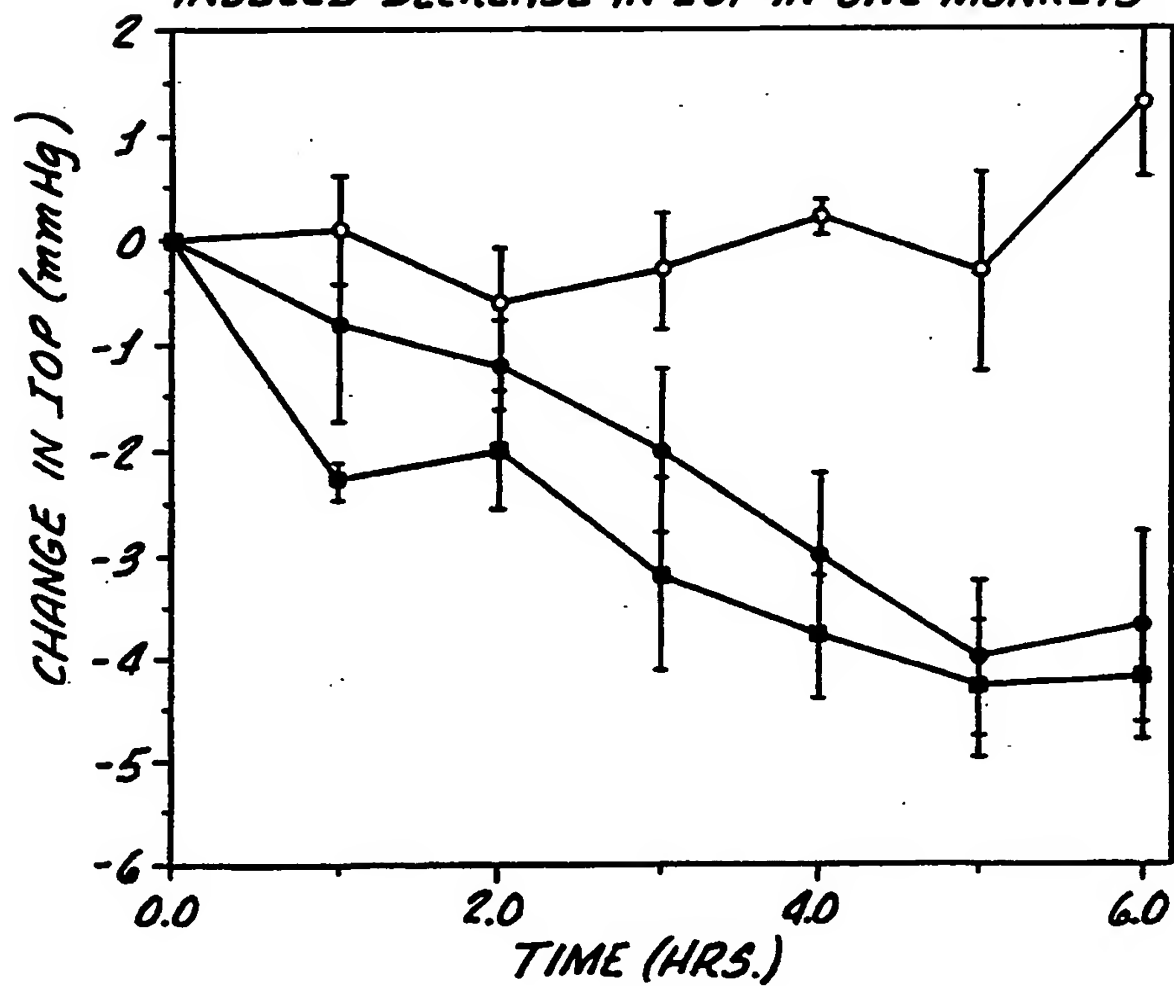


FIG. 7.

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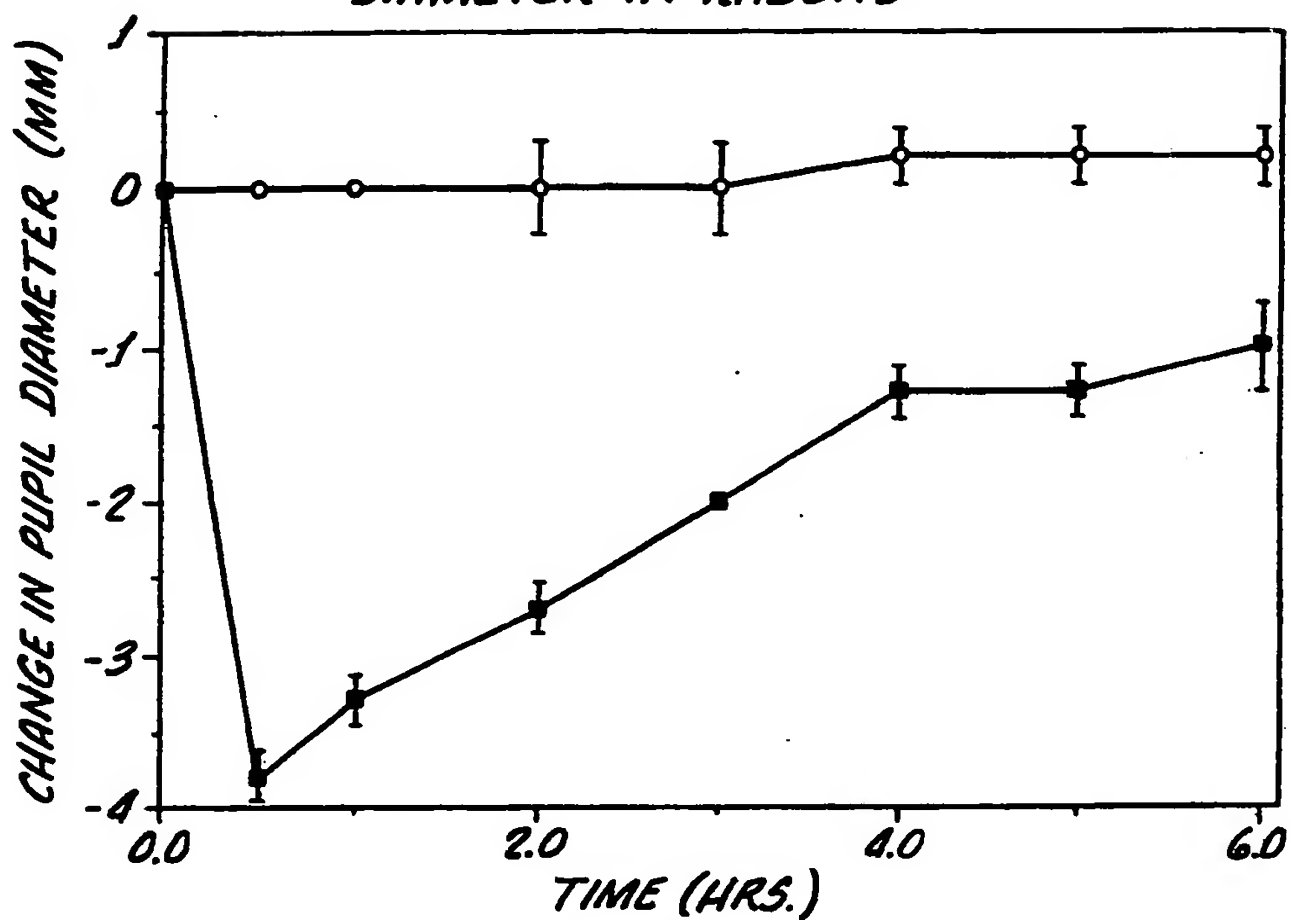
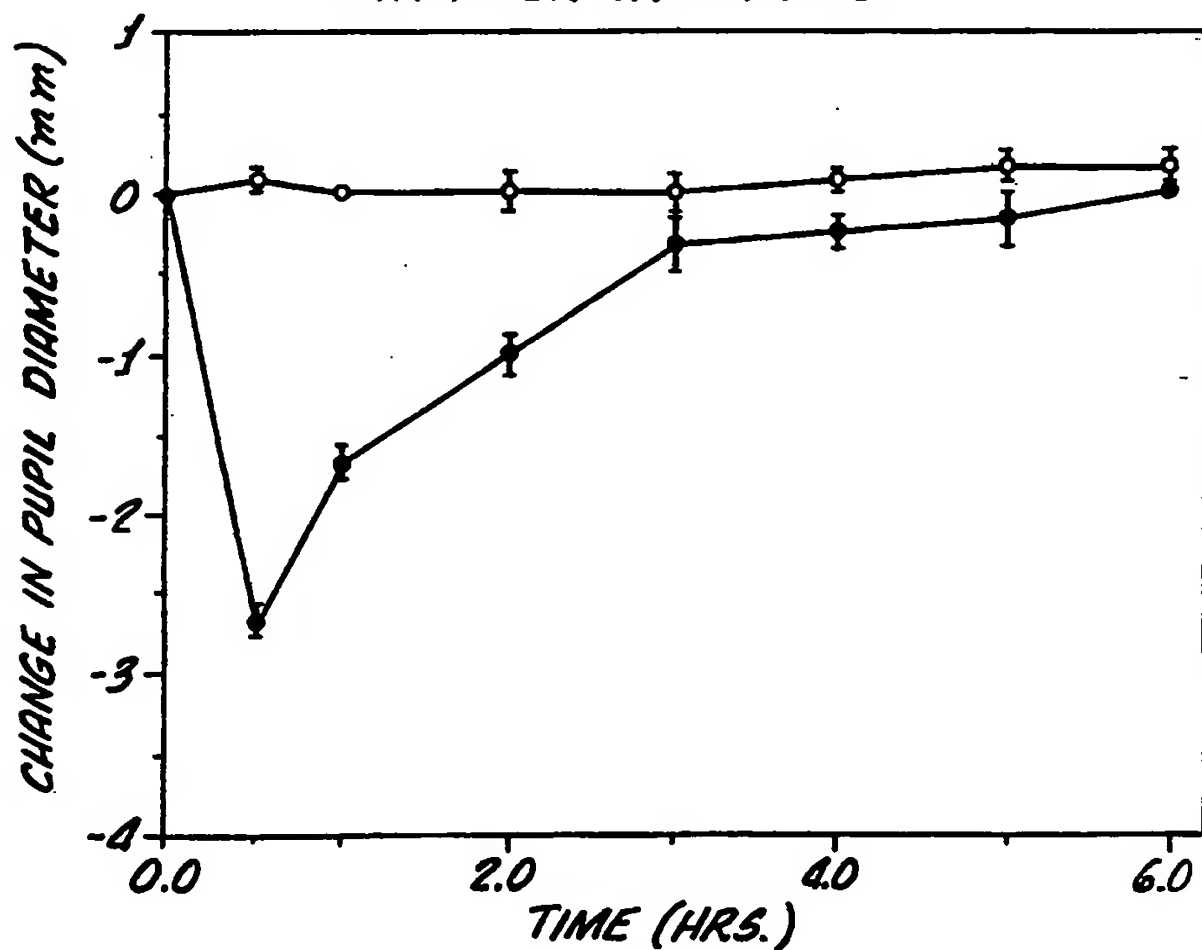
**EFFECT OF PILOCARPINE ON PUPIL
DIAMETER IN RABBITS**FIG. 8.**EFFECT OF TEST COMPOUND ON PUPIL
DIAMETER IN RABBITS**

Fig. 9.

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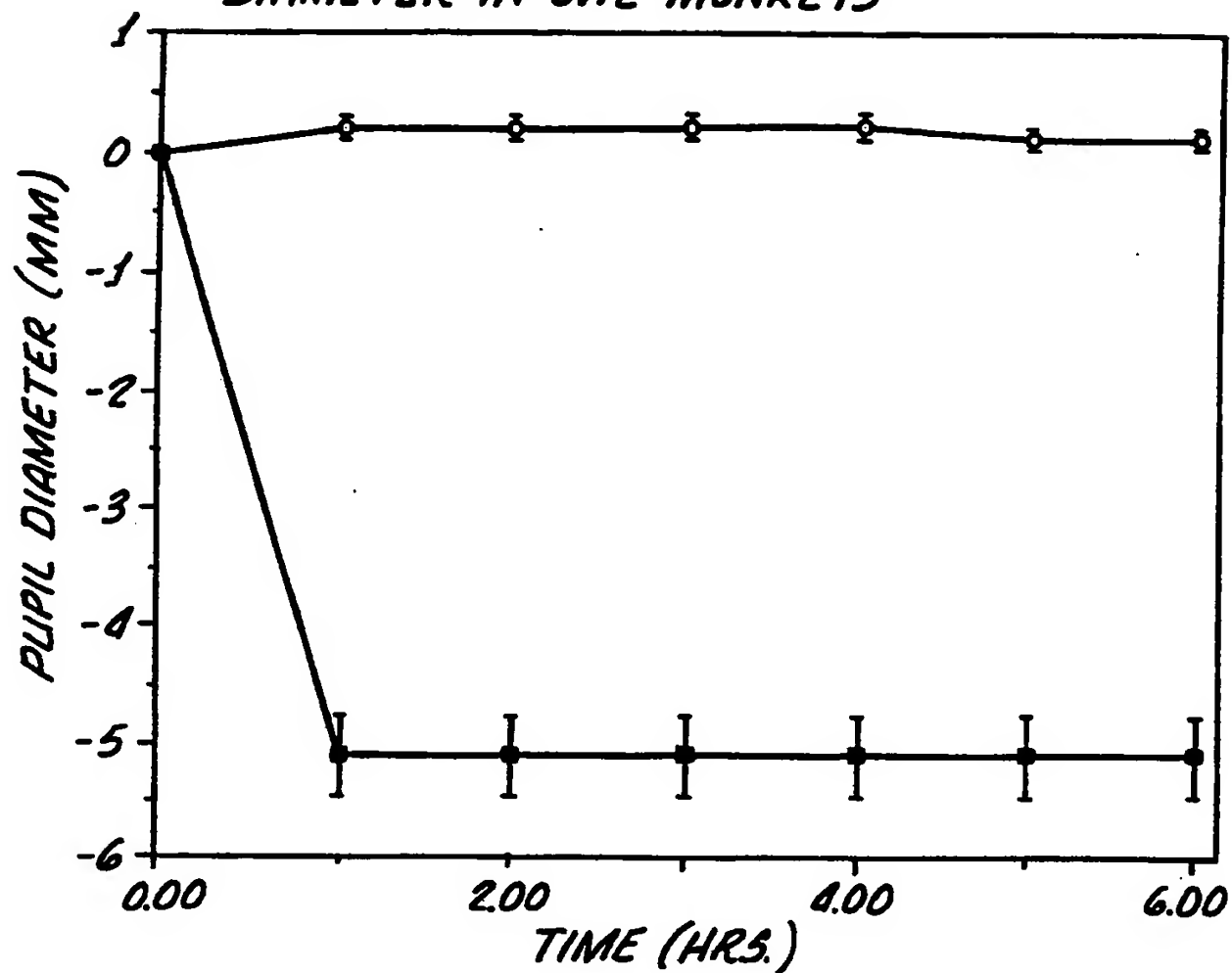
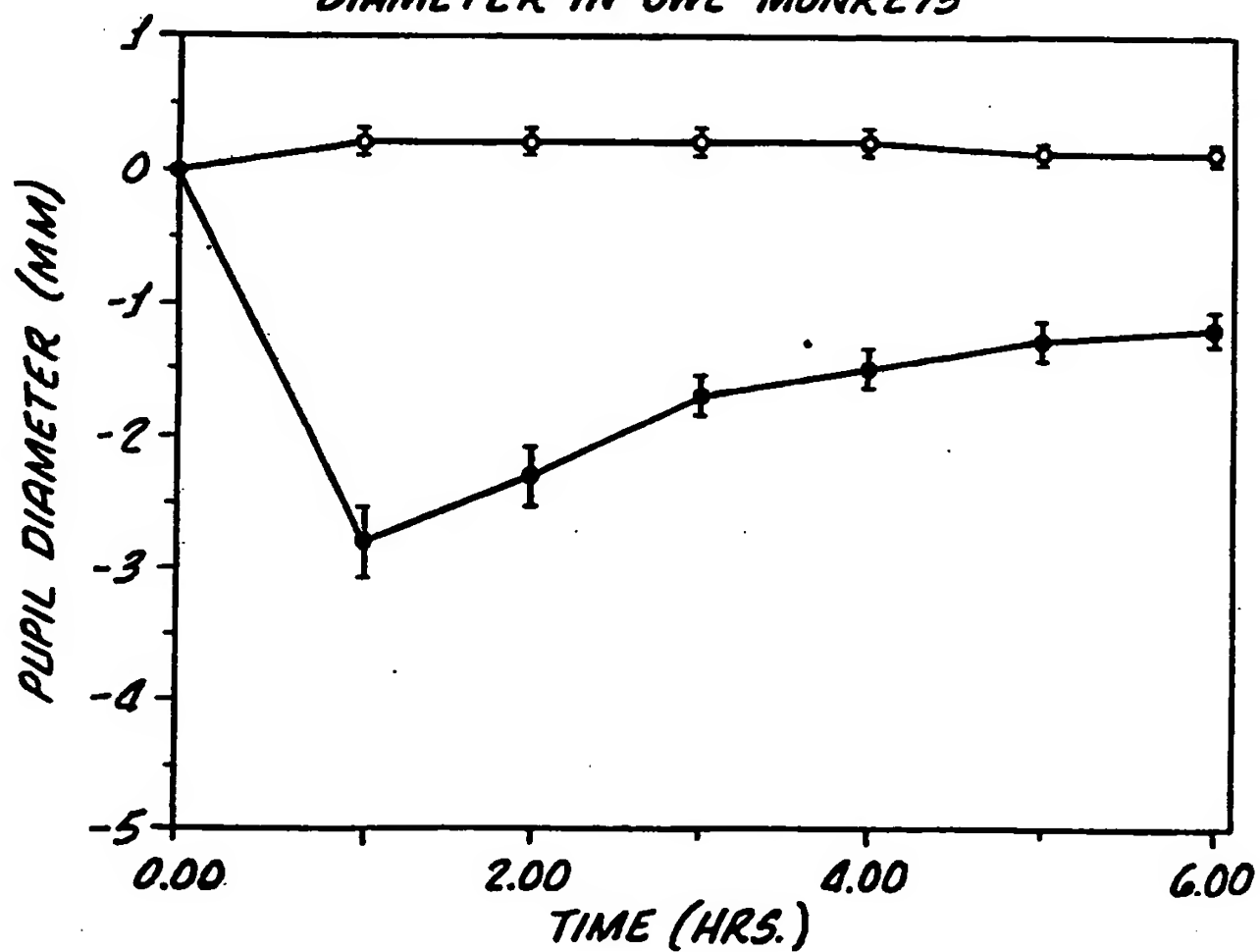
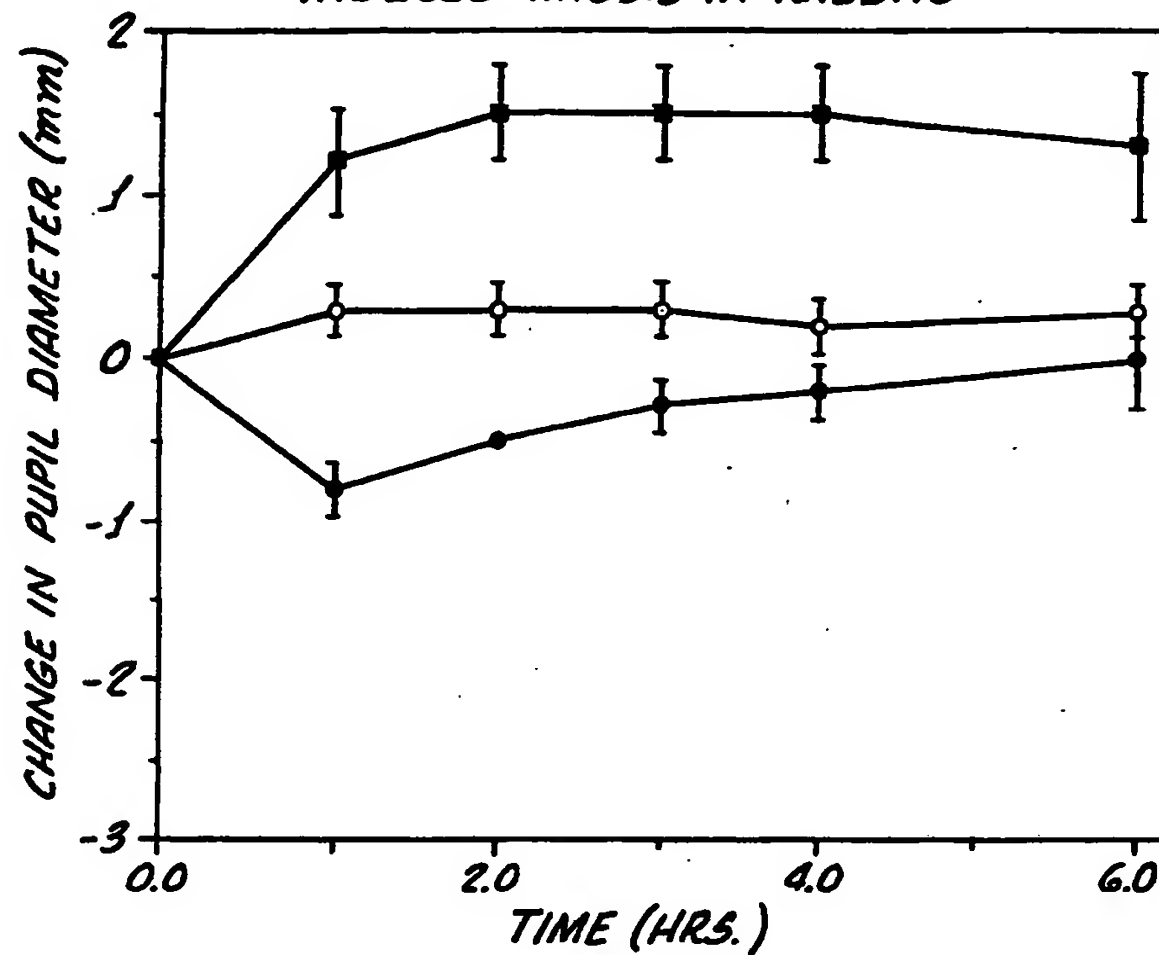
*EFFECT OF PILOCARPINE ON PUPIL
DIAMETER IN OWL MONKEYS*Fig. 10.*EFFECT OF TEST COMPOUND ON PUPIL
DIAMETER IN OWL MONKEYS*

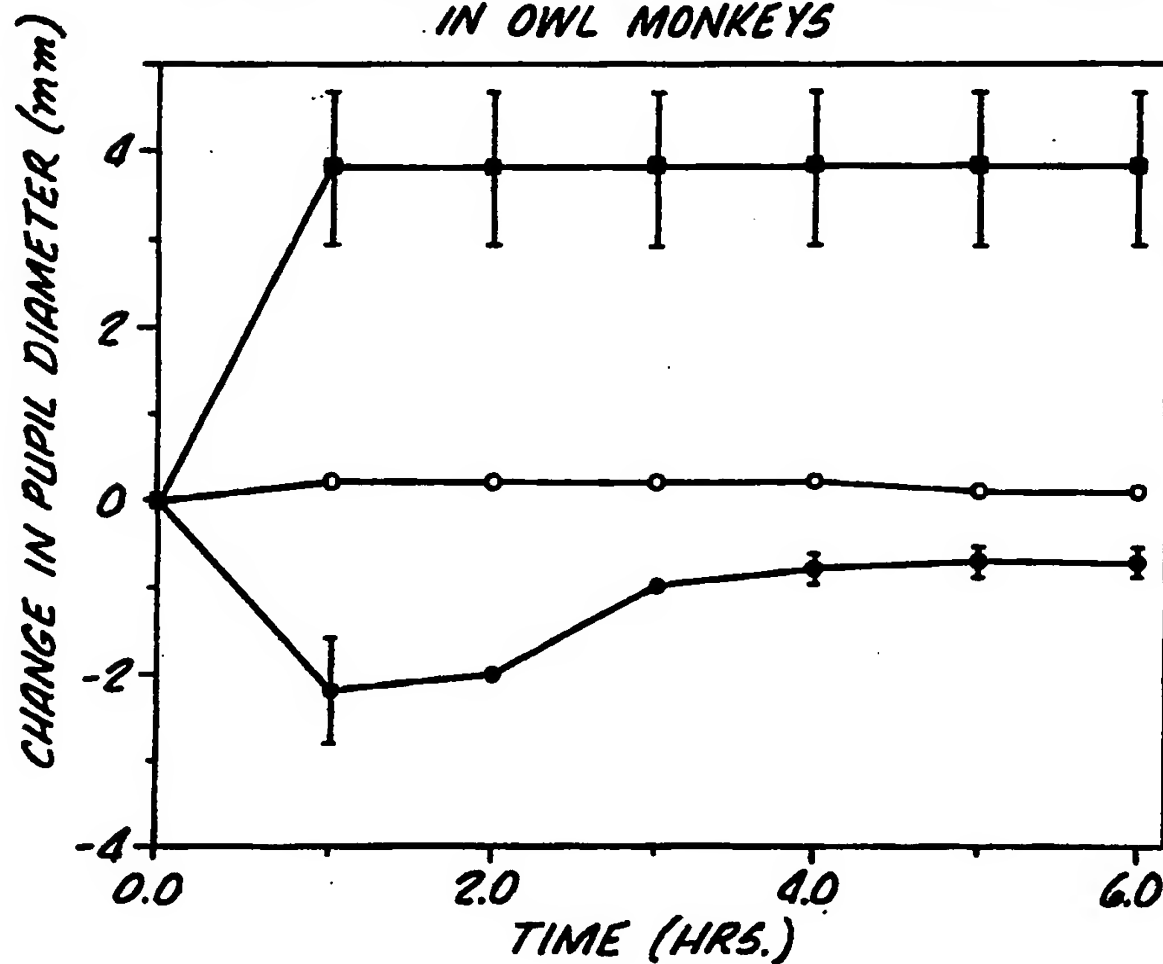
FIG. 11.

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EFFECT OF ATROPINE ON TEST COMPOUND
INDUCED MIOSIS IN RABBITS

FIG. 12.

EFFECT OF ATROPINE ON PUPIL DIAMETER
IN OWL MONKEYS



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CONTRACTION OF IRIS SPHINCTER OR
CILIARY MUSCLE BY TEST COMPOUND

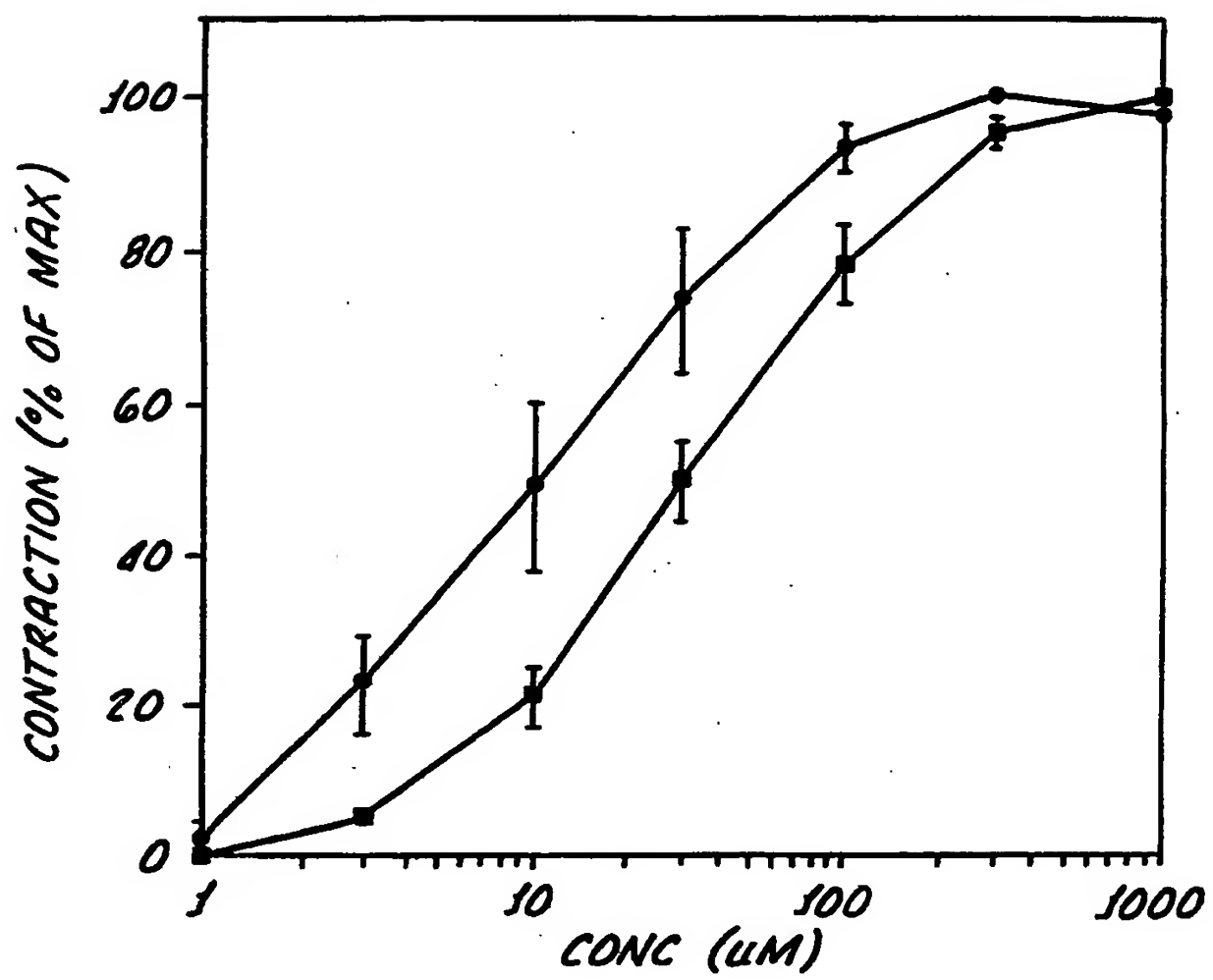
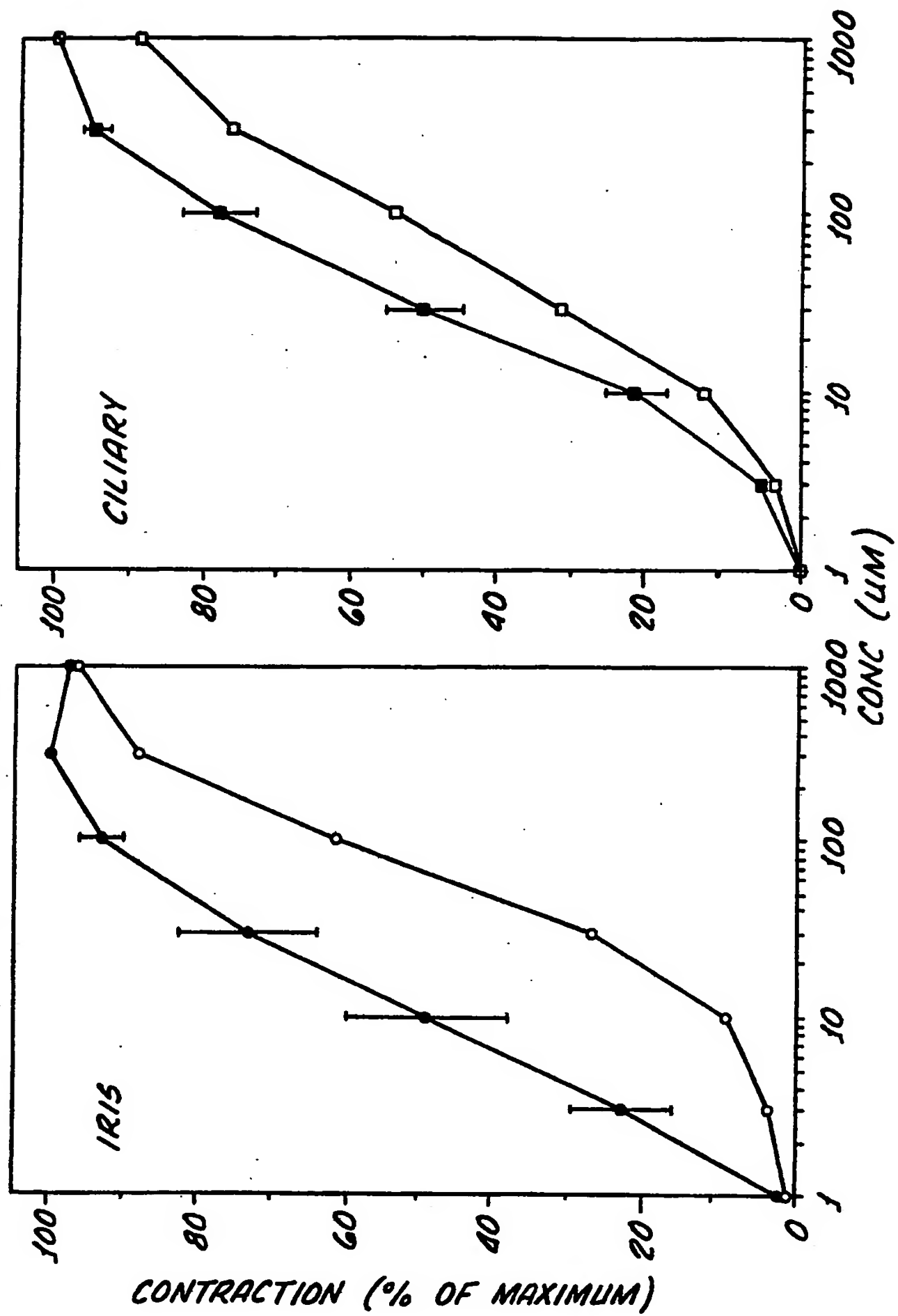


FIG. 13.

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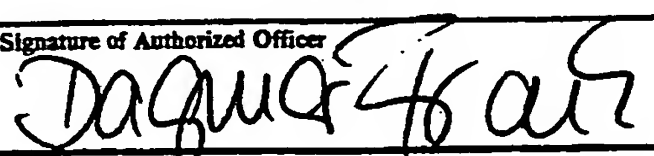
FIG. 14.

EFFECT OF ATROPINE ON TEST COMPOUND INDUCED
CONTRACTION IN IRIS OR CILIARY SMOOTH MUSCLE

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 91/08484

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.C1.5 A 61 K 31/435 A 61 K 31/44		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.C1.5	A 61 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EP,A,0384288 (A/S FERROSAN) 29 March 1990, see abstract; page 9, line 55 - page 10, line 17; example 1; claims ---	1-8,10, 11,13, 14,16, 17
Y	EP,A,0316718 (A/S FERROSAN) 24 May 1989, see abstract; page 3, line 21; example 18; claims (cited in the application) ---	1-18
Y	EP,A,0259621 (A/S FERROSAN) 16 March 1988, see abstract; page 3, line 21; examples 18,34,36; claims (cited in the application) ---	1-18
Y	EP,A,0296721 (H. LUNDBECK) 28 December 1988, see abstract; page 3, lines 32-34; claims (cited in the application) --- -/-	1-18
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
02-03-1992		24.04.92
International Searching Authority		Signature of Authorized Officer
EUROPEAN PATENT OFFICE		

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	Ph. DOROSZ: "Guide pratique des Médicaments", 7th edition, 1977, Maloine S.A., (Paris, FR), pages 816-817, see "Collyres antiglaucomateux: Pilocarpine, Acéclidine" ---	1-18
Y	EP,A,0307141 (MERCK SHARP & DOHME) 15 March 1989, see abstract; page 7, line 25; page 7, line 49 - page 8, line 5; claims ---	1-18
A	Journal of Medicinal Chem., vol. 33, no. 10, October 1990, L.J. STREET et al.: "Synthesis and biological activity of 1,2,4-oxadiazole derivatives: Highly potent and efficacious agonists for cortical muscarinic receptors", pages 2690-2697, see whole document ---	1-18
A	Journal of Medicinal Chem., vol. 33, no. 7, July 1990, A.M. MacLEOD et al.: "Synthesis and muscarinic activities of 1,2,4-thiadiazoles", pages 2052-2059, see whole document ---	1-18
E	EP,A,0459568 (MERCK SHARP & DOHME) 4 December 1991, see abstract; claims -----	1-10,13 ,16

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9108484
SA 54363

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 08/04/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0384288	29-08-90	AU-A- 4999690	30-08-90
		AU-A- 4999790	30-08-90
		CA-A- 2010578	22-08-90
		CA-A- 2010579	22-08-90
		EP-A- 0384285	29-08-90
		JP-A- 2255679	16-10-90
		JP-A- 2255680	16-10-90
		US-A- 5043345	27-08-91
		US-A- 5041455	20-08-91
EP-A- 0316718	24-05-89	AU-A- 2460888	18-05-89
		JP-A- 1153688	15-06-89
EP-A- 0259621	16-03-88	AU-B- 603603	22-11-90
		AU-A- 7816587	31-03-88
		JP-A- 63077877	08-04-88
		US-A- 4837241	06-06-89
		US-A- 4933353	12-06-90
		ZA-A- 8705550	08-02-88
EP-A- 0296721	28-12-88	AU-B- 615917	17-10-91
		AU-A- 1828288	05-01-89
		JP-A- 1022869	25-01-89
		US-A- 4866077	12-09-89
		US-A- 4925858	15-05-90
EP-A- 0307141	15-03-89	JP-A- 1151576	14-06-89
EP-A- 0459568	04-12-91	None	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

PLEASE SEE ADDITIONAL SHEET ../..

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1. SUBJECT-MATTER EXCLUDED FROM PATENTABILITY:

ALTHOUGH CLAIMS 1-12 ARE DIRECTED TO A METHOD OF TREATMENT OF THE HUMAN/ANIMAL BODY THE SEARCH HAS BEEN CARRIED OUT AND BASED ON THE ALLEGED EFFECTS OF THE COMPOUND/COMPOSITION.

2. OBSCURITIES, INCONSISTENCIES, LACK OF CONCISENESS, LACK OF READY COMPREHENSIBILITY:

IN VIEW OF THE LARGE NUMBER OF COMPOUNDS WHICH ARE DEFINED BY THE GENERAL FORMULA OF CLAIMS 1-2, THE SEARCH WAS LIMITED TO THE COMPOUNDS MENTIONED IN THE DESCRIPTION P. 6-7 (PCT ART. 6, GUIDELINES PART B, CHAPT. II.7. LAST SENTENCE AND CHAPT. III, 3.7).